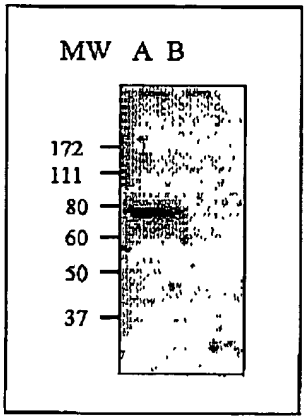
# APPENDIX

**EXPERIMENT A**
**Generation of rabbit Polyclonal Antibodies against Hi95 (Gene 95) Polypeptide**

Polyclonal anti human-Hi95 polylpeptide antibodies were generated by immunization of rabbits with a bacterially expressed fragment of the human Hi95 polypeptide encompassing amino acids 100-435. The polypeptide fragment contained an N-terminal histidine tag ($His_{10}$-$Hi95_{100-435}$ fusion protein). Prior to injection into rabbits, the antigen was affinity purified using Ni-columns. Testing of the antibodies generated can be seen in Figure 1.

**Figure 1**
Immunoblotting of Protein Lysates of Hi95 Infected Rat-1 Cells



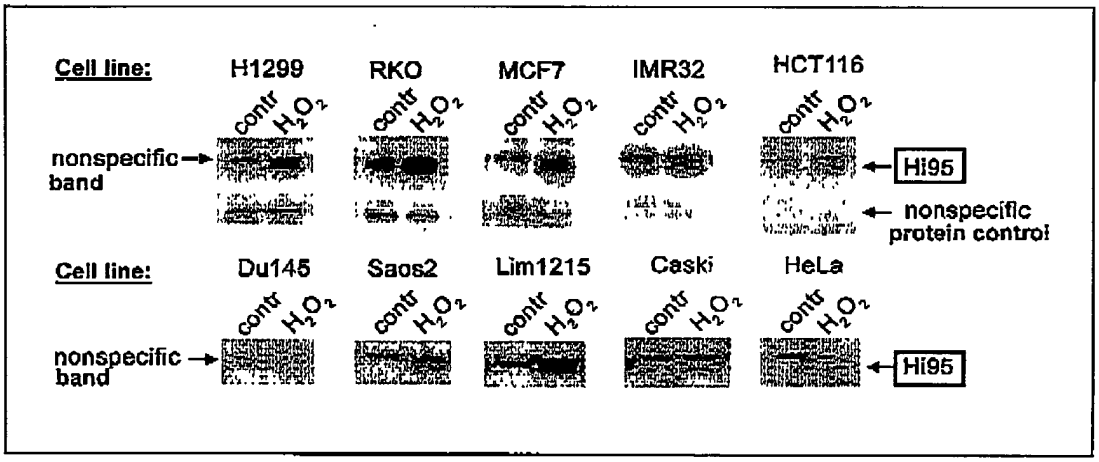

Immunoblotting of protein lysates of Hi95 infected Rat-1 cells 24 hours after infection (Lane A-10 µg/lane) and pBabe (empty vector) infected Rat-1 cells (Lane B -10 µg/lane), with αHP95 (lot #AP30902301) 1 µg/ml. MW markers: kDa.

**EXPERIMENT B**

**Expression of the Human Hi95 Polypeptide Is Induced by Oxidative Stress in Various Human Cell Lines**

A number of different human cancer cell lines were treated with 0.5 M $H_2O_2$ for 24 hours *in vitro* or left untreated for the same period of time. Total protein extracts were separated on 10% polyacrylamide gels, transferred to nitrocellulose membrane and immunoblotted with anti-Hi95 antibodies. The results of these experiments can be seen in Figure 2.

Figure 2
Immunoblotting of Protein Extracts Derived from Various Cell Lines before and after Oxidative Stress Treatment with Anti-Hi95 Antibodies



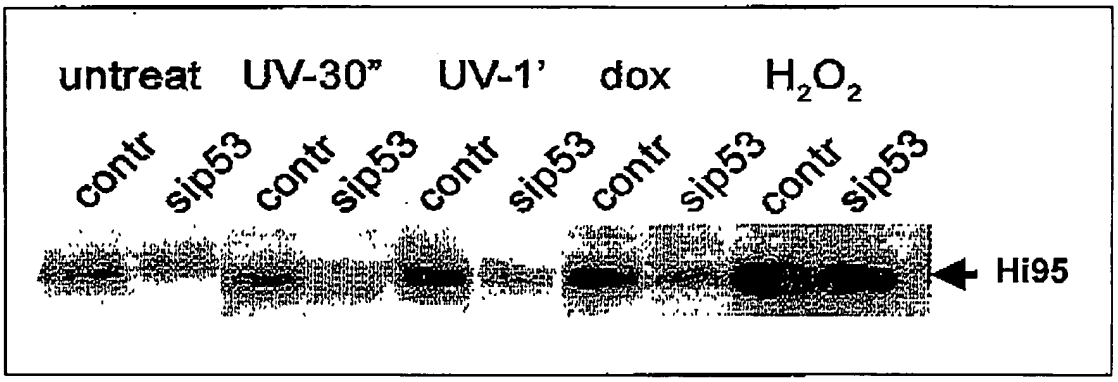
**EXPERIMENT C**

**Expression of the Hi95 Polypeptide Is Induced in Response to DNA Damage in a p53-Dependent Manner**

P53-positive RKO cells were infected with a lentiviral vector expressing shRNA against p53 or with an empty vector as a control. Isogenic cell pairs were subjected to doxorubicin (50 ng/ml) or UV treatment, which confer genotoxic stress. Induction of Hi95 polypeptide expression was completely abolished in the RKO cells in which p53 expression was inactivated via RNA interference. In contrast, both types of cells retained the ability to strongly induce Hi95 protein in response to $H_2O_2$. The results of these experiments are demonstrated in Figure 3.

**Figure 3**

Immunoblotting of Protein Extracts Derived from Isogenic p53-Positive and p53-Negative RKO Cells before and after Genotoxic stress Treatments with anti-Hi95 Antibodies




Immunoblotting with anti-Hi95 antibodies of protein extracts from isogenic p53-positive and p53-negative RKO cells.

Contr – RKO cells infected with empty lentiviral vector. sip53 - RKO cells infected with lentivirus expressing shRNA against p53. The treatments are indicated over the blot.